

Week 1 (06/06 – 10/06)

1. Transformation

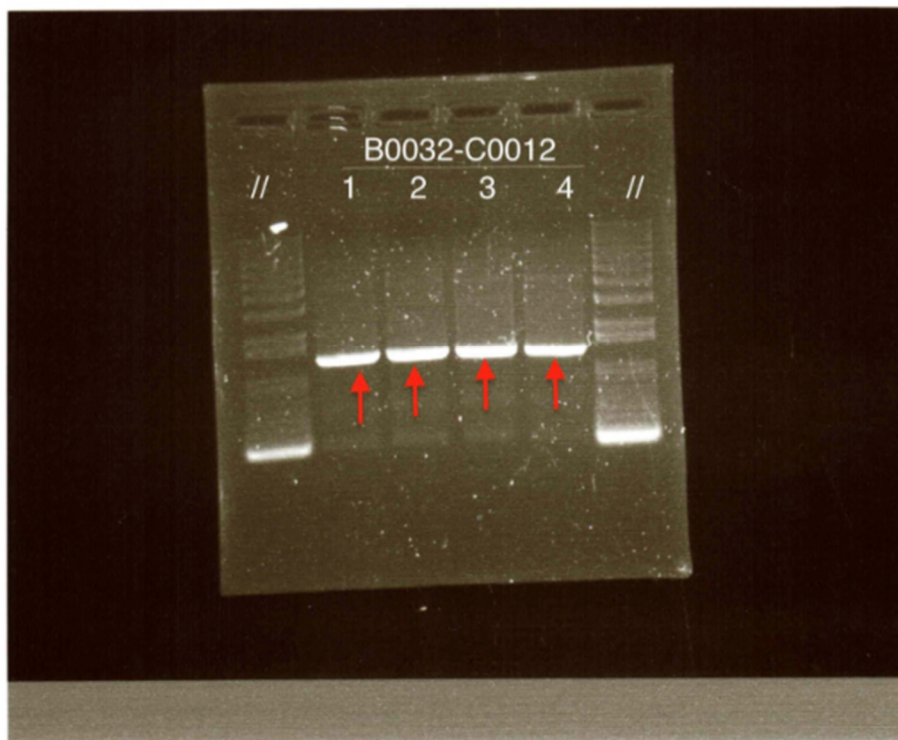
- pSB1C3-BBa_R0010 (*lacp*)
- pSB1C3-BBa_C0012 (LacI)
- pSB1C3-BBa_S0100 (RBS-LacI)
- pSB3K3-BBa_E0240 (GFP generator)

2. Phusion PCR of BBa_B0032-C0012 (RBS-LacI)

Desired band size: 1333bp

- Sample: 3221 bp
- Observed band sizes in gel photo: Between 1000 bp and 1650 bp

6/10/2016, 10:03 AM; Size: 1392x1032; Exp: 5380ms; Bin: 1x1; Modif: No; Disp BWG: (305, 25285, 1.
File: n/a (unsaved)



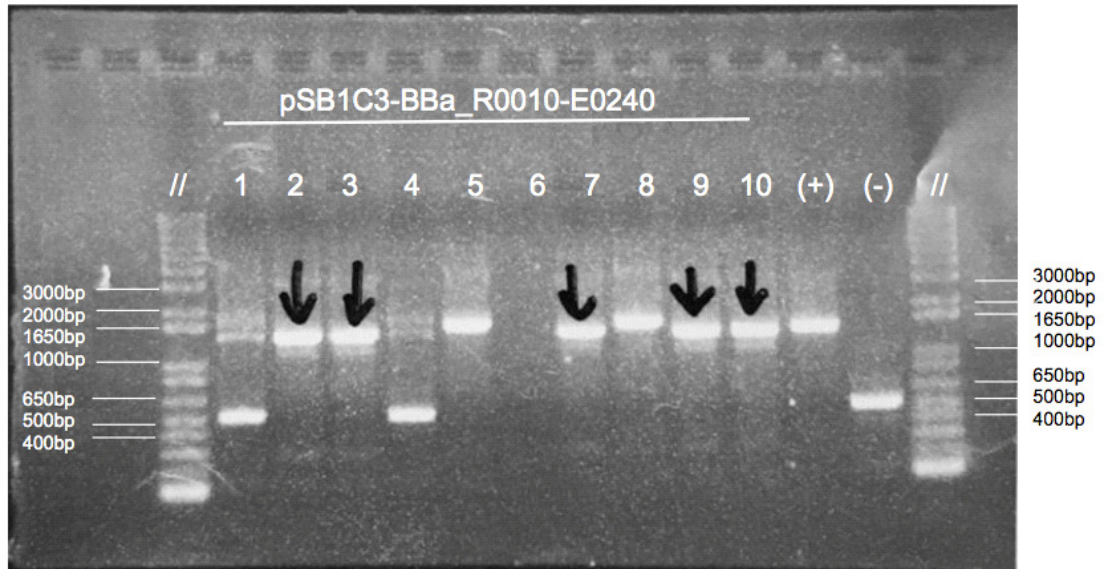
3. Digestion and Ligation for pSB1C3-BBa_R0010-E0240

Week 2 (13/06-17/06)

1. Colony PCR of pSB1C3-BBa_R0010-E0240

Desired band size: 1398bp

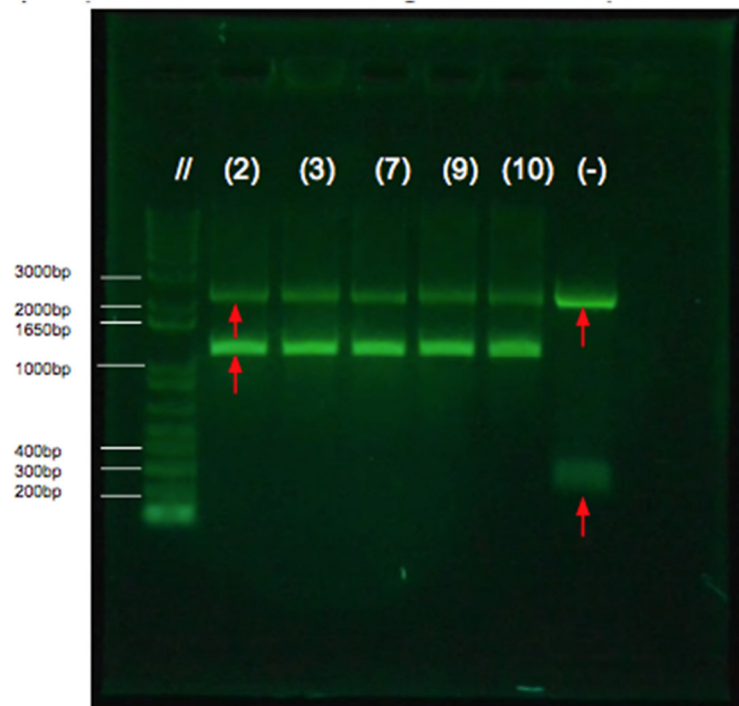
- Sample: 3154 bp
- Negative control (pSB1C3-BBa_R0010): 514 bp
- Observed band sizes in gel photo: Between 1000 bp and 1650 bp



2. Restriction Check of pSB1C3-BBa_R0010-E0240

Desired band sizes: 2029 and 1125bp

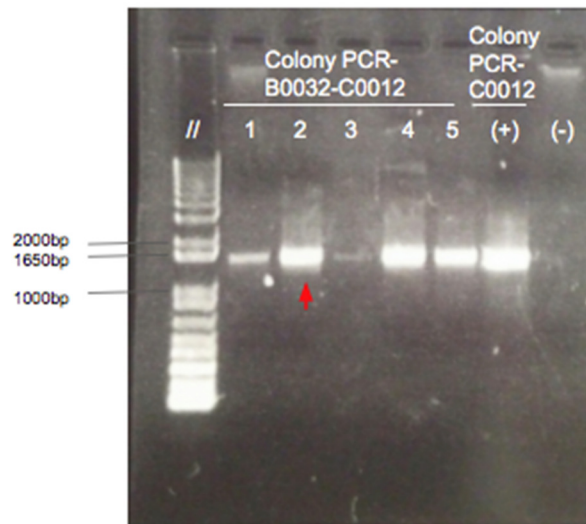
- Sample: 3154 bp
- Negative control (pSB1C3-BBa_R0010): 2029 and 241 bp
- Observed band sizes in gel photo: Between 2000 and 3000 bp, 1000 and 1650 bp



3. Colony PCR of pSB3K3-BBa_B0032-C0012

Desired band sizes: 1453bp

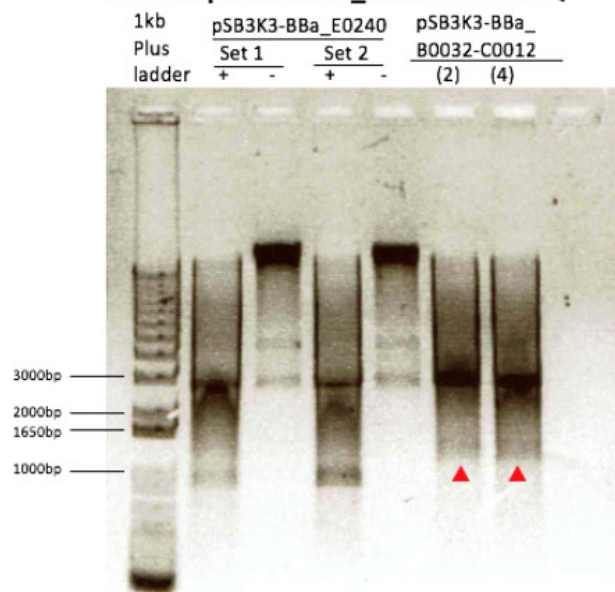
- Sample: 3922 bp
- Observed band sizes in gel photo: Between 1000 and 1650 bp



Week 3 (20/06 – 24/06)

1. Transfer of BBa_R0010-E0240 from pSB1C3 to pSB3K3

Digestion of pSB3K3-BBa_E0240 and Restriction Check of pSB3K3-BBa_B0032-C0012MQ



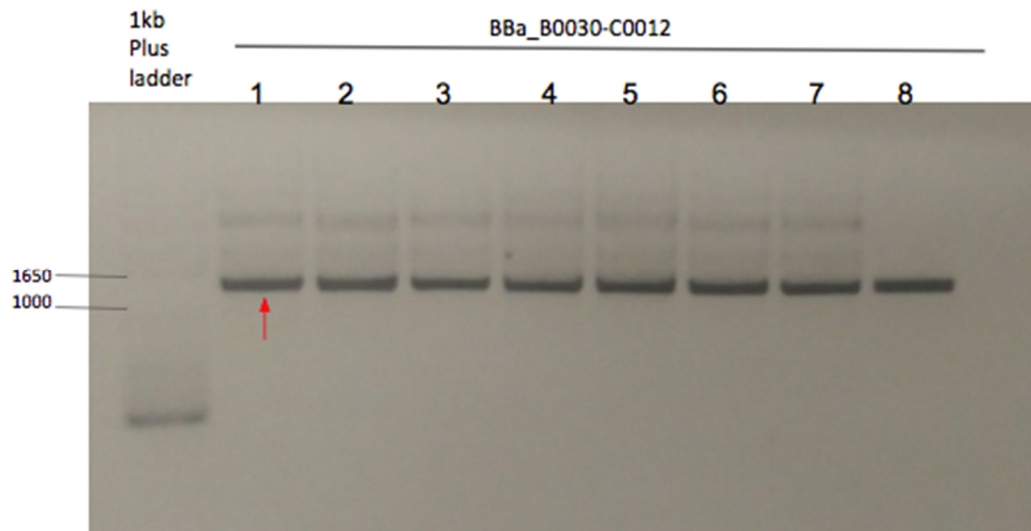
Digestion of pSB3K3-BBa_E0240 and Restriction Check of pSB3K3-BBa_B0032-C0012. 18µl of samples are mixed with 2µl of 2.5X loading dye. DNA was resolved in 0.7% gel. Visualisation was done by pre-staining with Midori Green Advance DNA stain. Red triangles indicate the correct band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

2. Phusion PCR of BBa_B0030-C0012

Desired band sizes: 1361bp

- Sample: 3244 bp

- Observed band sizes in gel photo: Between 1000 and 1650 bp

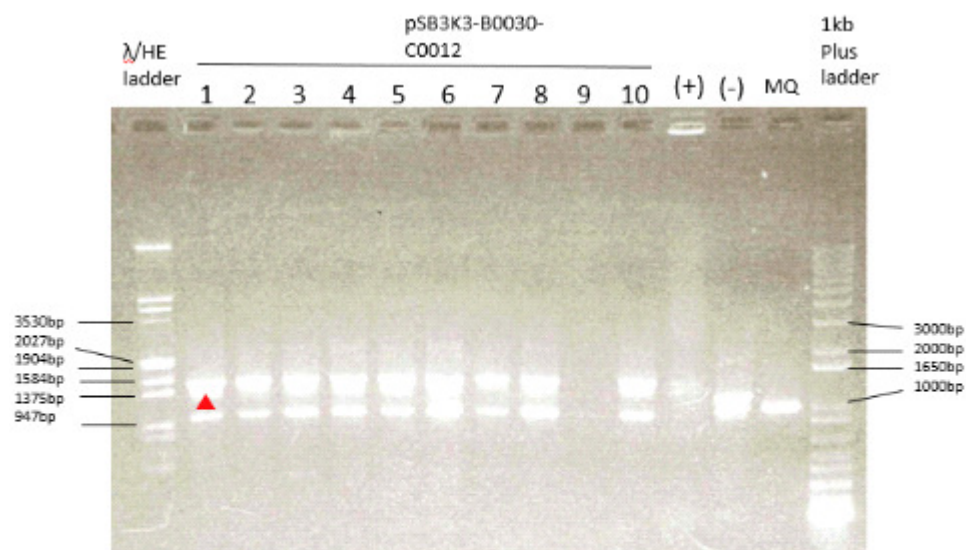


3. Colony PCR of pSB3K3-BBa_B0030-C0012

Desired band sizes: 1361bp

- Sample: 3924 bp

- Observed band sizes in gel photo: Between 1000 and 1650 bp



Colony PCR of pSB3K3-B0030-C0012. 18µl of samples are mixed with 2µl of 2.5X loading dye. DNA was resolved in 0.7% gel. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the correct band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

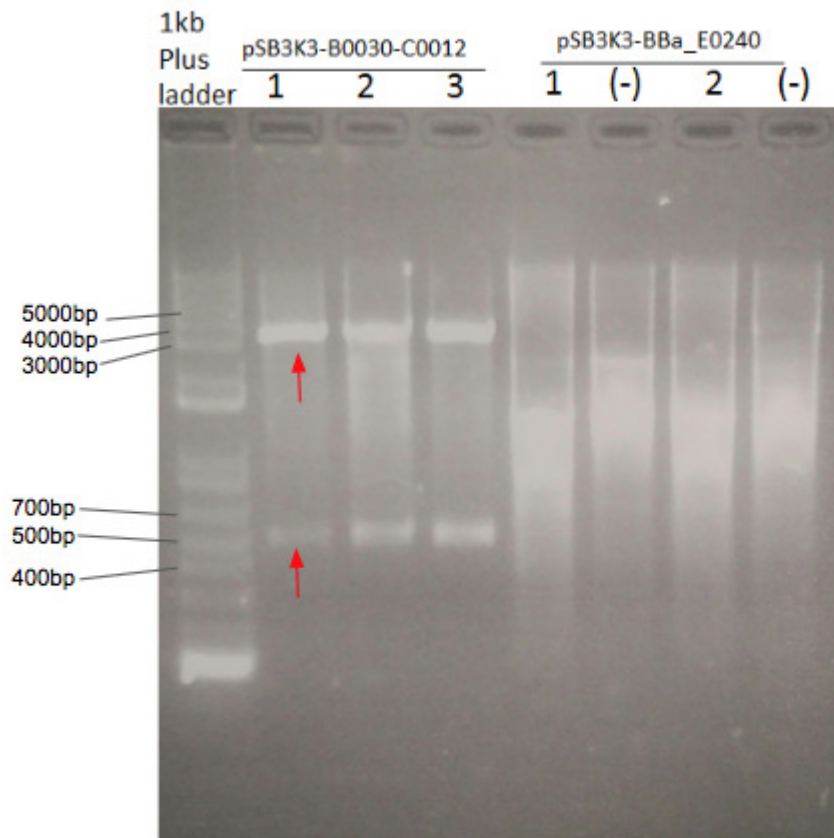
4. Restriction Check of pSB3K3-BBa_B0030-C0012 with AseI

Desired band sizes: 529 and 3395bp

- Sample: 3244 bp

- Observed band sizes in gel photo: Between 500 and 650 bp, 3000 and 4000 bp

Restriction check of pSB3K3-B0030-C0012



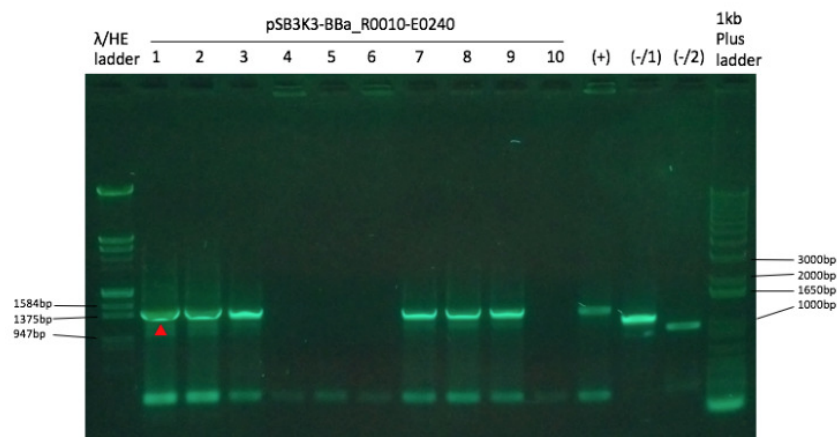
Restriction check of pSB3K3-B0030-C0012

18µl of samples are mixed with 2µl of 2.5X loading dye. DNA was resolved in 0.7% gel. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the correct band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 4 (27/06 – 01/07)

1. Colony PCR of pSB3K3-BBa_R0010-E0240

Colony PCR of pSB3K3-BBa_R0010-E0240 with VF₂ and VR

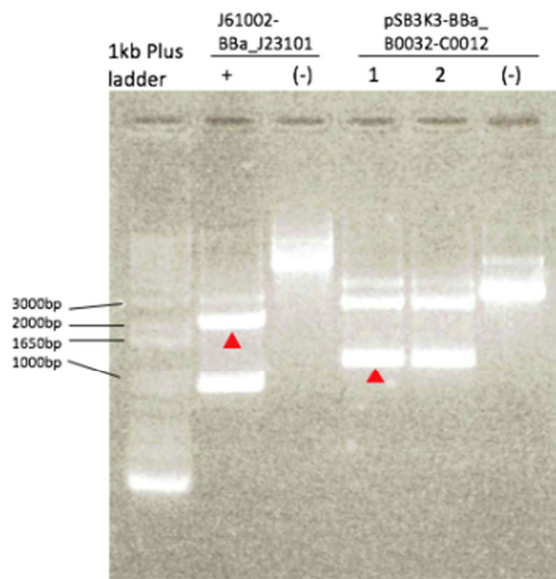


Colony PCR of pSB3K3-BBa_R0010-E0240 with VF₂ and VR. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 0.7% gel. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicate the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 5 (04/07 – 08/07)

1. Digestion and Ligation of pSB1A2-BBa_J23101-B0032-C0012

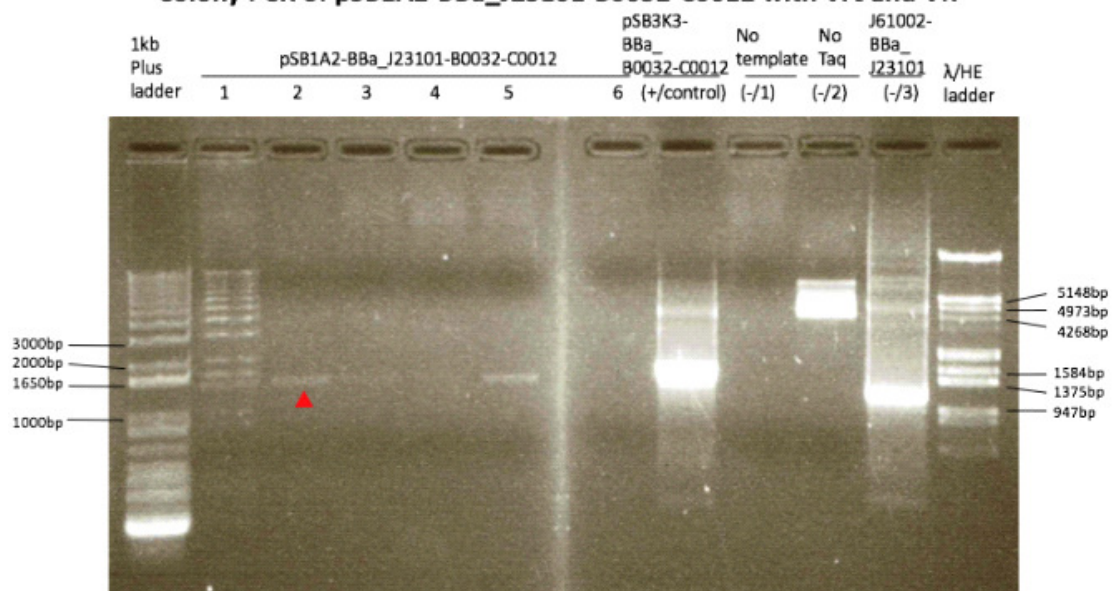
Digestion of pSB3K3-BBa_B0032-C0012 and J61002-BBa_J23101



Digestion of pSB3K3-BBa_B0032-C0012 and J61002-BBa_J23101. J61002-BBa_J23101 is digested with SpeI-HF and PstI-HF and pSB3K3-BBa_B0032-C0012 is digested with XbaI and PstI-HF restriction enzymes. 18 μ l of samples are mixed with 2 μ l of 10X loading dye. DNA was resolved in 1% gel at 6.67V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the correct band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

2. Colony PCR of pSB1A2-BBa_J23101-B0032-C0012

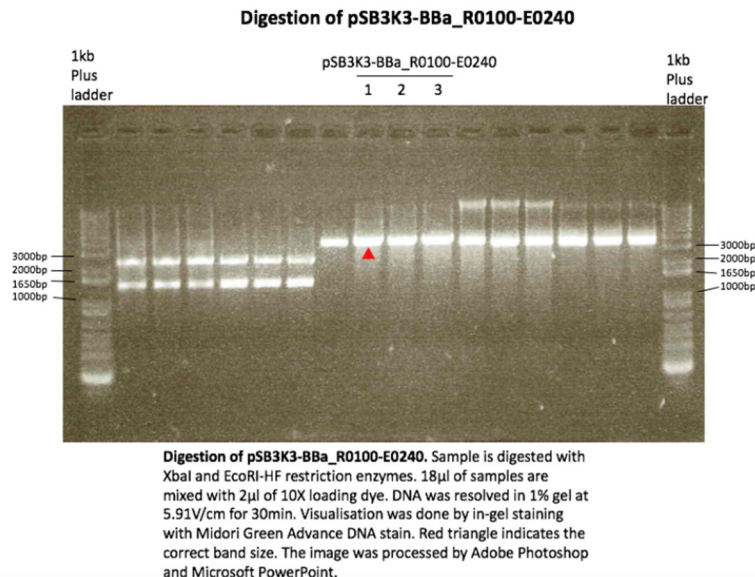
Colony PCR of pSB1A2-BBa_J23101-B0032-C0012 with VF₂ and VR



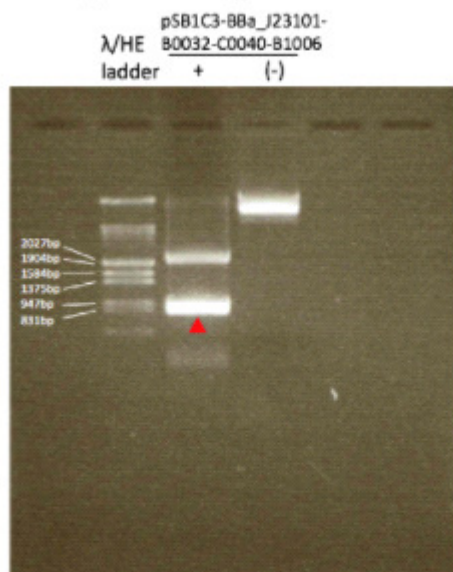
Colony PCR of pSB1A2-BBa_J23101-B0032-C0012 with VF₂ and VR. 18 μ l of samples are mixed with 2 μ l of 10X loading dye. DNA was resolved in 0.7% gel. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicate the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

3. Restriction Check of pSB1A2-BBa_J23101-B0032-C0012 with EcoRI-HF and PstI-HF and Digestion of pSB1C3-BBa_B1006

4. Digestion and ligation for pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240

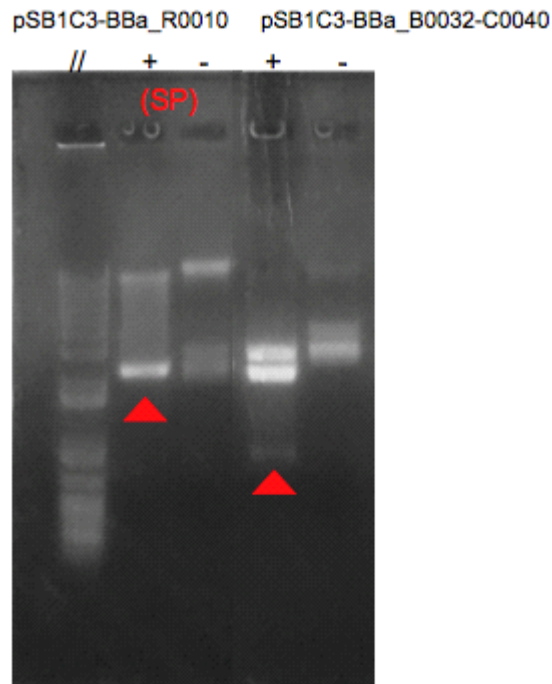


Digestion of pSB1C3-BBa_J23101-B0032-C0040-B1006



Digestion of pSB1C3-BBa_J23101-B0032-C0040-B1006. Sample is digested with XbaI and EcoRI-HF restriction enzymes. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.41V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

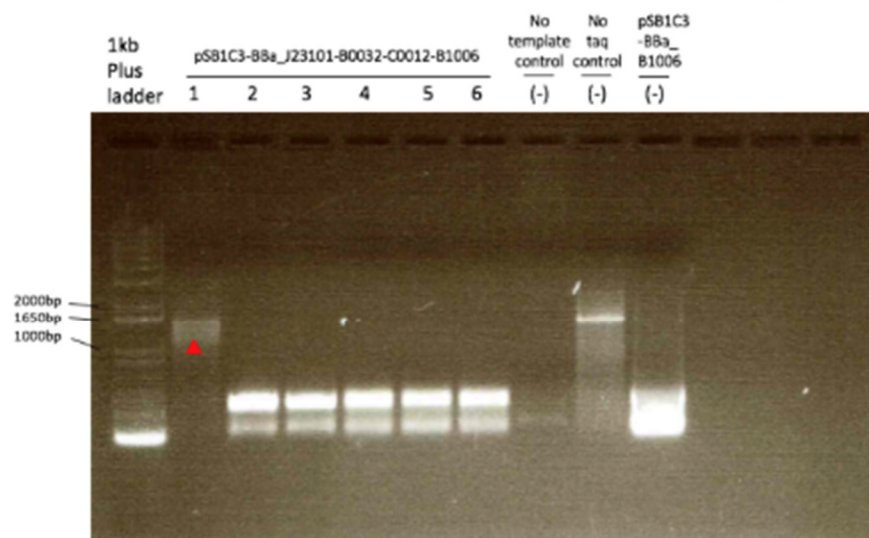
5. Digestion and Ligation for pSB1C3-BBa_R0010-B0032-C0040



Week 6 (11/07 - 15/07)

1. Colony PCR of pSB1C3-BBa_J23101-B0032-C0012-B1006 with VF₂ and VR

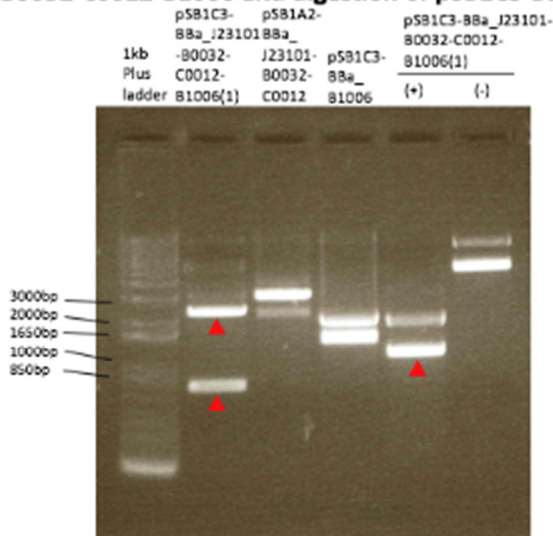
Colony PCR of pSB1C3-BBa_J23101-B0032-C0012-B1006 with VF₂ and VR



Colony PCR of pSB1C3-BBa_J23101-B0032-C0012-B1006 with VF₂ and VR. 25µl of samples were mixed with 2.78µl of 10X loading dye. DNA was resolved in 1% gel at 7.64V/cm for 45min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

2. Restriction Check of pSB1C3-BBa_J23101-B0032-C0012-B1006 with StyI-HF

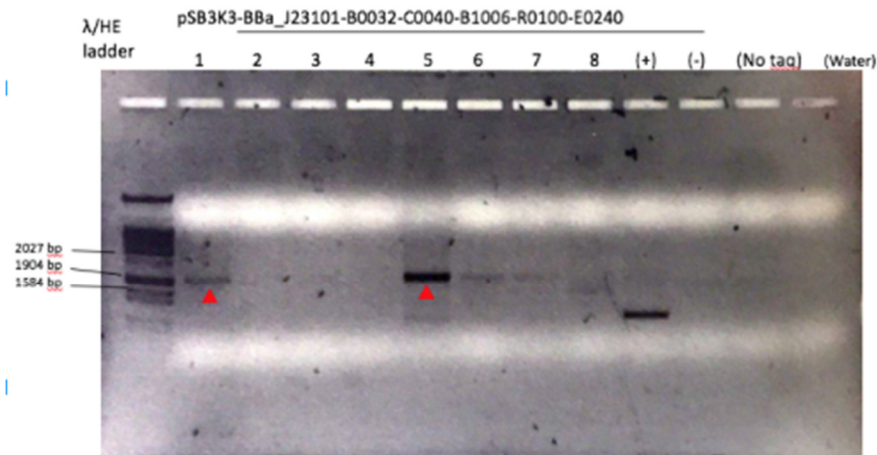
Restriction check and digestion of pSB1C3-BBa_J23101-B0032-C0012-B1006 and digestion of pSB1C3-BBa_B1006



Restriction check and digestion of pSB1C3-BBa_J23101-B0032-C0012-B1006 and digestion of pSB1C3-BBa_B1006. pSB1C3-BBa_J23101-B0032-C0012-B1006 was digested with Styl-HF for restriction check and with EcoRI-HF and SpeI-HF for ligation. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.5V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the correct band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

3. Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240

Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0100-E0240 with J23101FW and VR

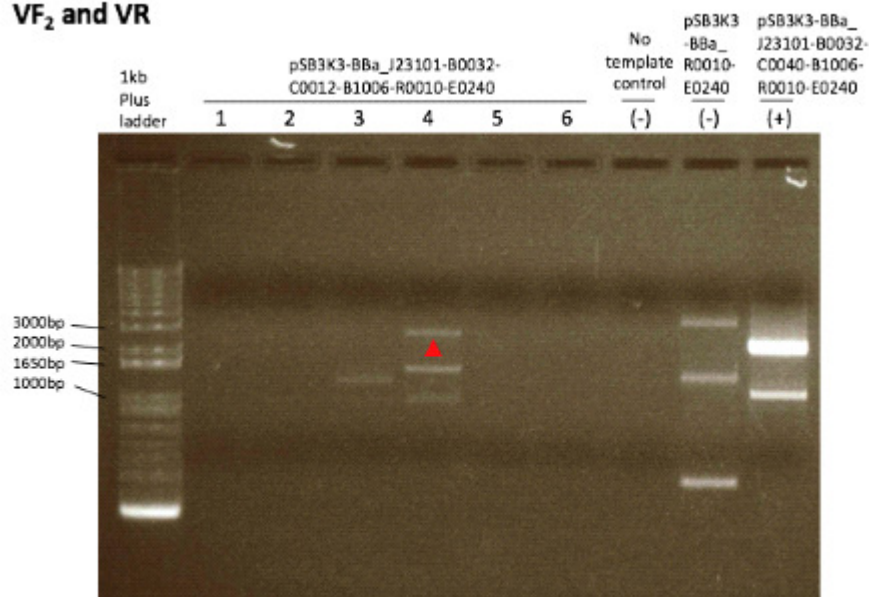


Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0100-E0240 with J23101FW and VR. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.41V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 7 (18/07 – 22/07)

1. Colony PCR of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0010-E0240

Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240 with VF₂ and VR

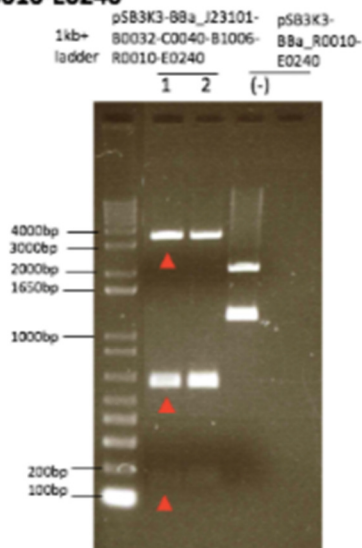


Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240 with VF₂ and VR.

25µl of samples were mixed with 2.78µl of 10X loading dye. DNA was resolved in 1.025% gel at 7.27V/cm for 25min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

2. Restriction Check of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240

Restriction check of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240

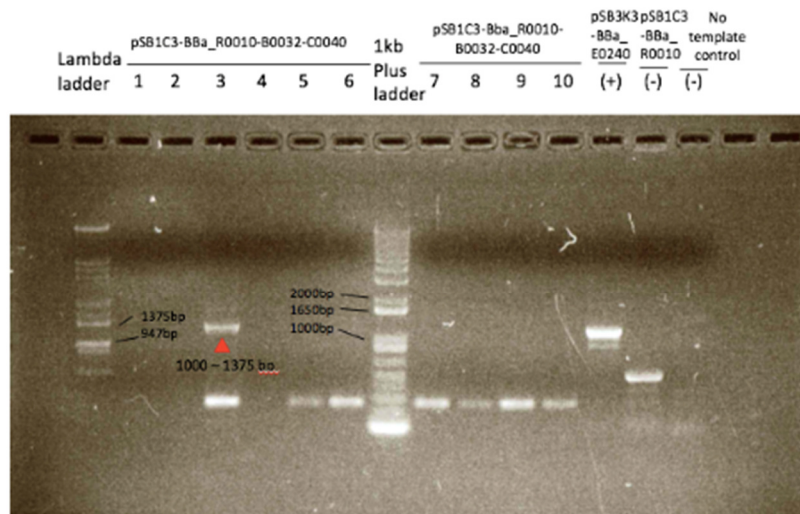


Restriction check of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240.

Sample is digested with Styl-HF restriction enzymes. 18µl of samples are mixed with 2µl of 5X loading dye. DNA was resolved in 1.5% gel at 7.27V/cm for 45min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

3. Colony PCR of pSB1C3-BBa_R0010-B0032-C0040

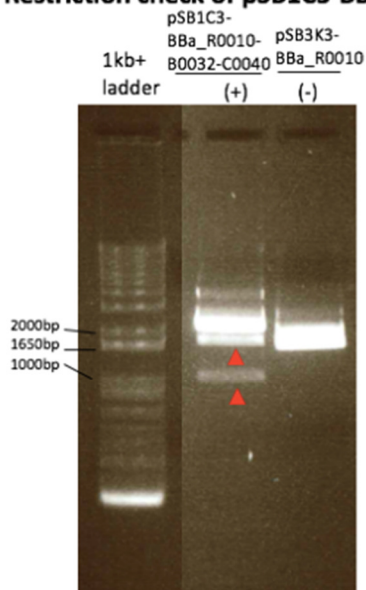
Colony PCR of pSB1C3-BBa_R0010-B0032-C0040 with VF₂ and VR



Colony PCR of pSB1C3-BBa_R0010-B0032-C0040 with VF₂ and VR. 18µl of samples were mixed with 2µl of 5X loading dye. DNA was resolved in 1% gel at 7.27V/cm for 40min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the desired band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

4. Restriction Check of pSB1C3-BBa_R0010-B0032-C0040

Restriction check of pSB1C3-BBa_R0010-B0032-C0040



Digestion of pSB1C3-BBa_R0010-B0032-C0040(3). Sample is digested with ScaI-HF restriction enzymes. 18µl of samples are mixed with 2µl of 5X loading dye. DNA was resolved in 1.025% gel at 7.27V/cm for 25min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 8 (25/07 - 03/08)

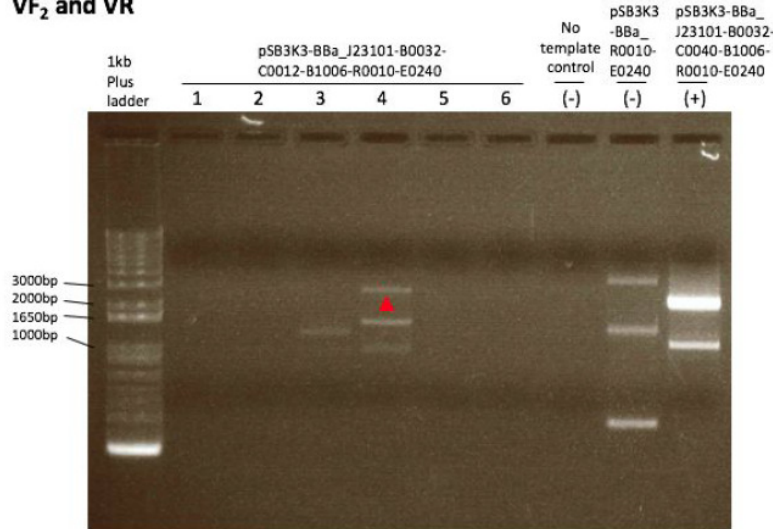
1. Colony PCR of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0010-E0240 with VR and VF₂

Desired band sizes:

- Sample: 2683 bp

- Negative control : 1400 bp
- Observed band sizes in gel photo: Between 2000 bp and 3000 bp, 1000 bp and 1650 bp

Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240 with VF₂ and VR



Colony PCR of pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240 with VF₂ and VR.
 25µl of samples were mixed with 2.78µl of 10X loading dye. DNA was resolved in 1.025% gel at 7.27V/cm for 25min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

- PCR of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0010-E0240 with J23101 FW and VR
 - Desired band sizes:
 Sample: 2543 bp
 Positive control : 2062 bp
 - Observed band sizes in gel photo:
 - Between 2000 bp and 3000 bp

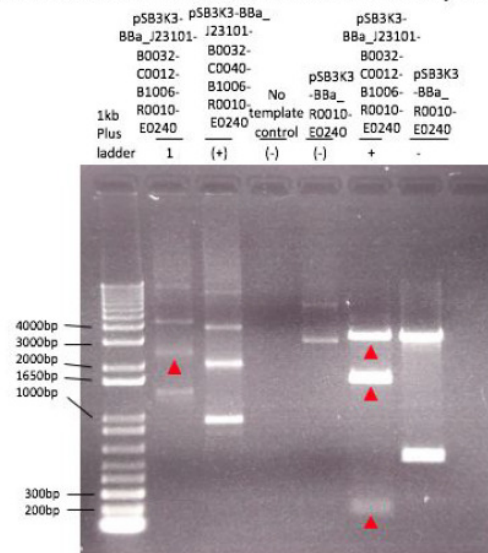
3. Restriction check of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0010-E0240 with Styl-HF

- Desired band sizes:

Sample: 3296bp, 1658bp and 163bp

Negative control: 3296bp and 538bp

PCR and Restriction Check of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0010-E0240 with J23101 FW and VR and with Styl-HF



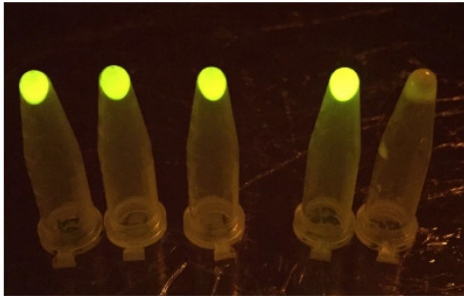
PCR of and Restriction Check of pSB3K3-BBa_J23101-B0032-C0012-B1006-R0010-E0240 with J23101 FW and VR and with Styl-HF. 18µl of samples were mixed with 2µl of 10X loading dye. DNA was resolved in 1.1% gel at 5.91V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the desired band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 9 (4/8 – 11/8)

1. Characterization of Construct A and Construct B

- Log 10 intervals was used between 0 to 5×10^3 uM IPTG for both constructs.
- For construct A, the difference between adding 5×10^3 uM IPTG and no IPTG is not significant.
- However, construct A expresses fluorescence(compared with the GFP generator) after shaking in
- the 37C incubator for 2 hours and 05 mins, contrary to results from shaking for 1 hour and 05
- mins in the 37C incubator.

2. Functional Assay of Construct A



Construct A (+1) (+7) (+9) (+c)(-c)

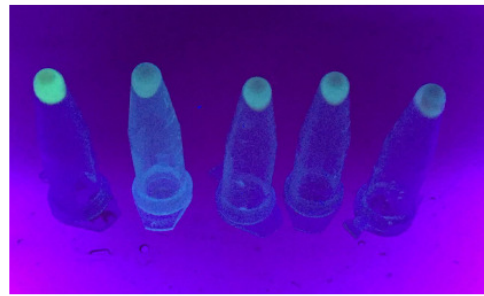
Positive control:

Construct A of Ptet group

Negative control:

pSB3K3-BBa_E0240

Functional Assay for Construct A -
Inoculated overnight with 5mM IPTG
viewed under blue light and filtered
with an orange filter.



Construct A (+1) (+7) (+9) (+c)(-c)

Positive control:

Construct A of Ptet group

Negative control:

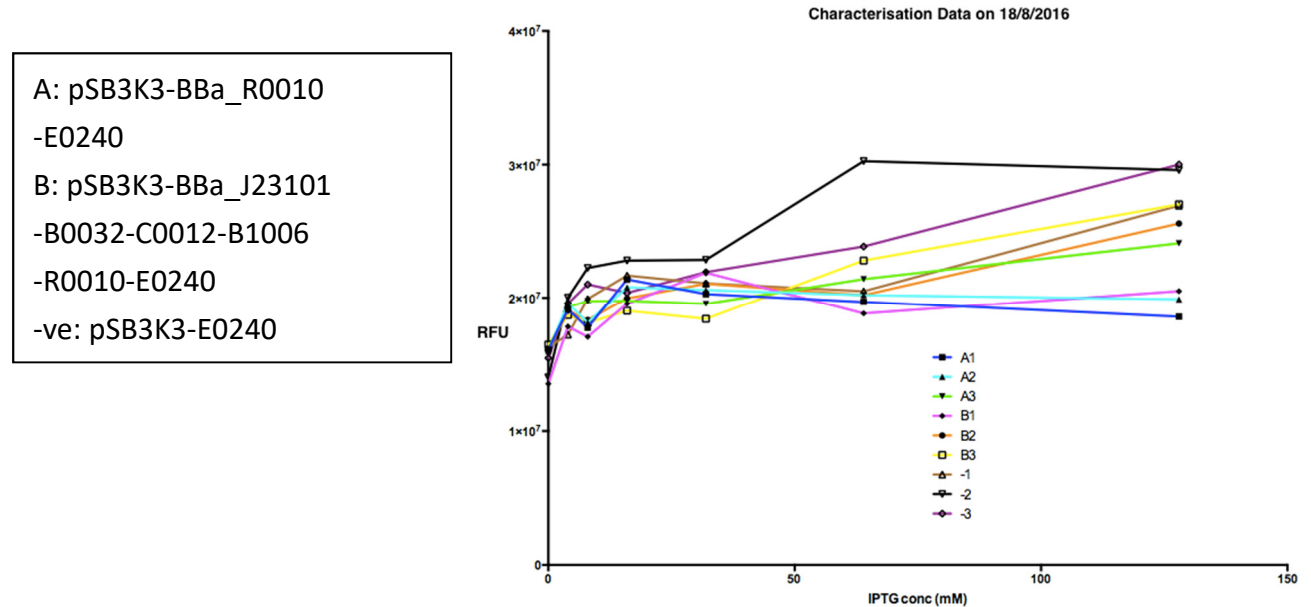
pSB3K3-BBa_E0240

Functional Assay for Construct A -
Inoculated overnight with 5mM IPTG
viewed under UV light machine.

Week 10 (12/8-19/8)

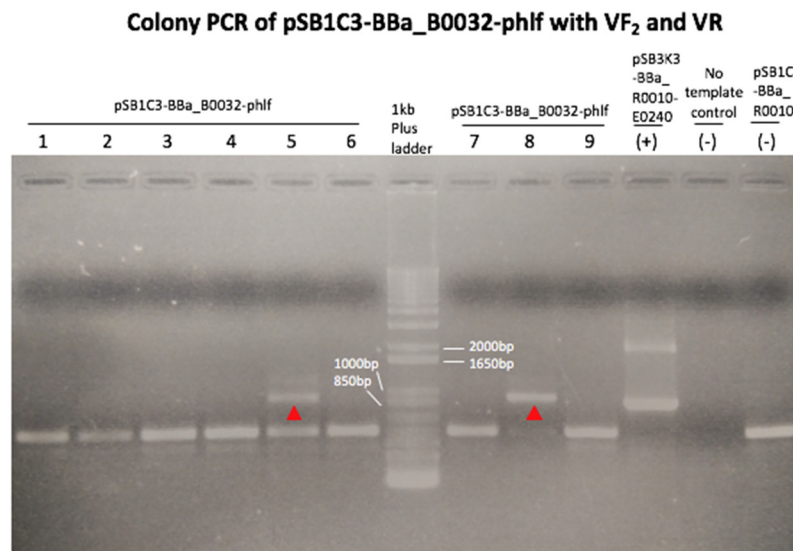
1. Characterization of Construct A and B on 18/8/2016

- Samples with inducers were shaken overnight.
- Then a functional assay was done.
- Samples were diluted to 0.5 OD 600 before loaded to the 96 wells plate and read by Envision.



2. Colony PCR of pSB1C3-BBa_B0032-phlF

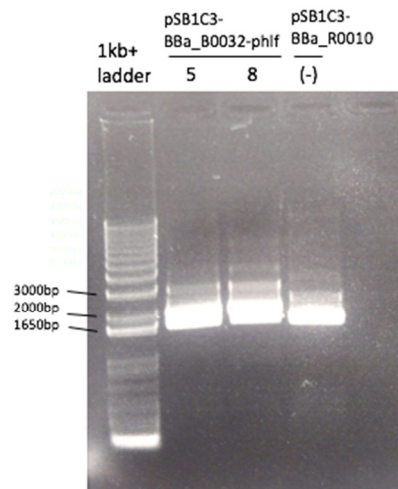
- Desired band: 936bp
- Inoculated colonies 5 and 8



Colony PCR of pSB1C3-BBa_B0032-phlF with VF₂ and VR. 25µl of samples were mixed with 2.78µl of 10X loading dye. DNA was resolved in 1% gel at 5.91V/cm for 35min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate desired band sizes. The image was processed by Microsoft PowerPoint.

3. Restriction check of pSB1C3-BBa_B0032-phlF with Scal-HF
 - Desired bands: 1796 bp and 896 bp

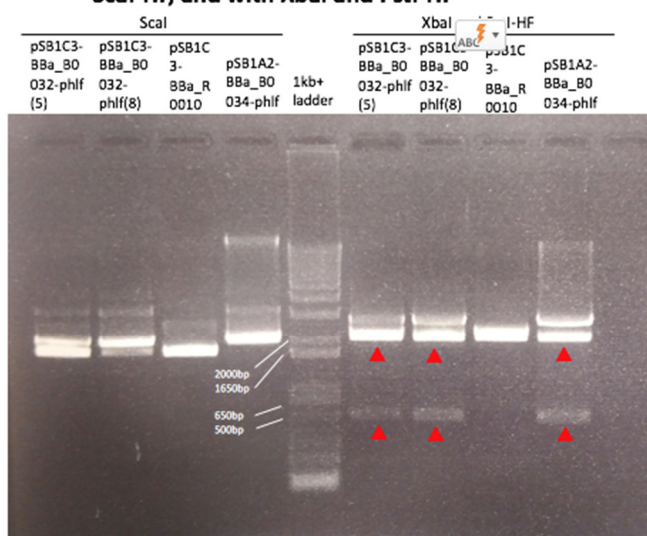
Restriction check of pSB1C3-BBa_B0032-phlF with Scal-HF



Restriction check of pSB1C3-BBa_B0032-phlF with Scal-HF. Samples were digested with Scal-HF restriction enzyme. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.67V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. The image was processed by Microsoft PowerPoint.

4. Restriction check of pSB1C3-BBa_B0032-phlF
 - Set 1: Scal-HF
 - Desired bands: 1796 bp and 896 bp
 - Set 2: XbaI and PstI-HF
 - Desired bands: 2044 and 648bp

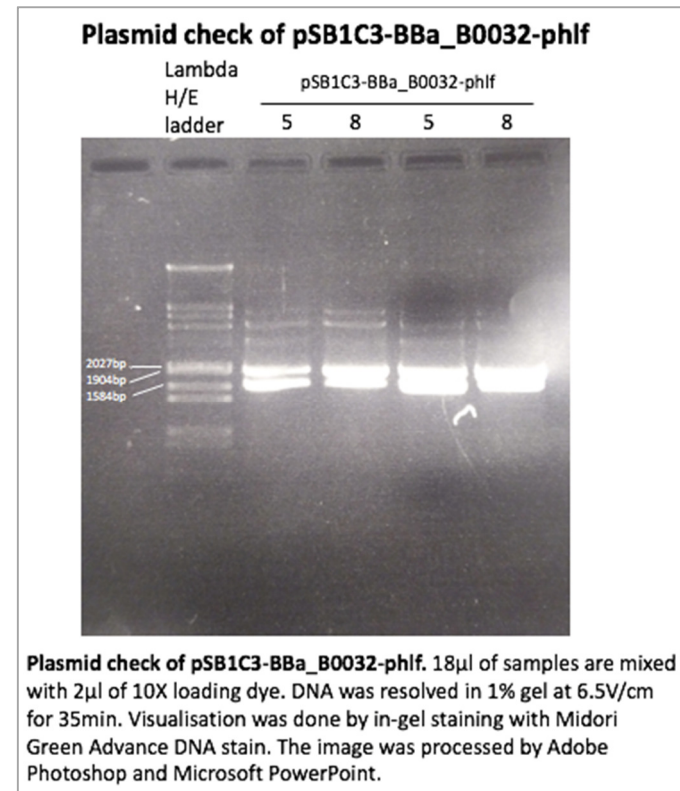
Restriction check of pSB1C3-BBa_B0032-phlF with Scal-HF, and with XbaI and PstI-HF



Restriction check of pSB1C3-BBa_B0032-phlF. Samples were digested with Scal-HF, and with XbaI and PstI-HF restriction enzymes. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 5.91V/cm for 35min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

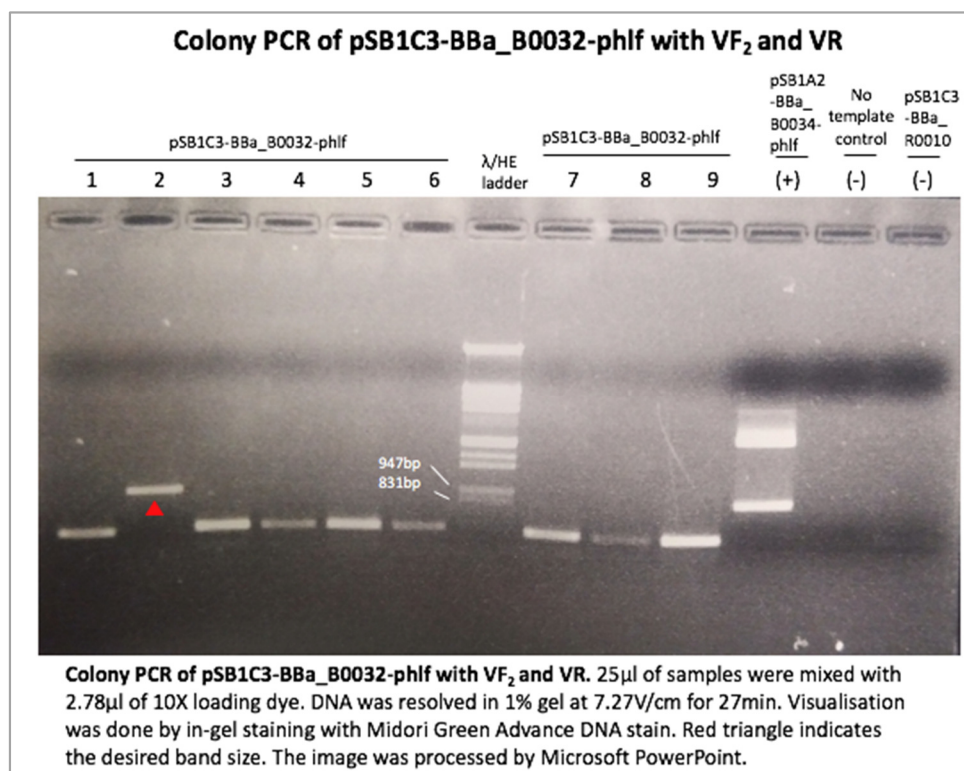
5. Plasmid check of pSB1C3-BBa_B0032-phlF

- Plasmid band size: 2692bp
- Backbone band size: 2270bp



6. Colony PCR of pSB1C3-BBa_B0032-phlF

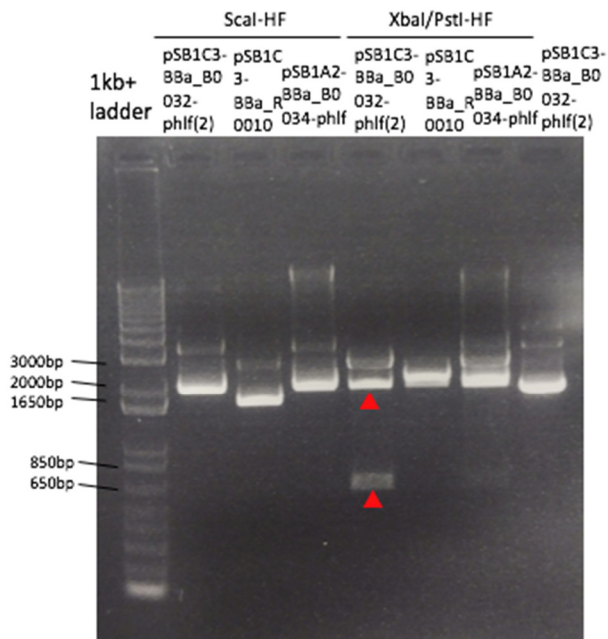
- Desired band: 936bp
- Inoculated colony 2



7. Restriction check of pSB1C3-BBa_B0032-phlF

- Set 1: Scal-HF
- Desired bands: 1796 bp and 896 bp
- Set 2: XbaI and PstI-HF
- Desired bands: 2044 and 648bp
- Send to sequencing

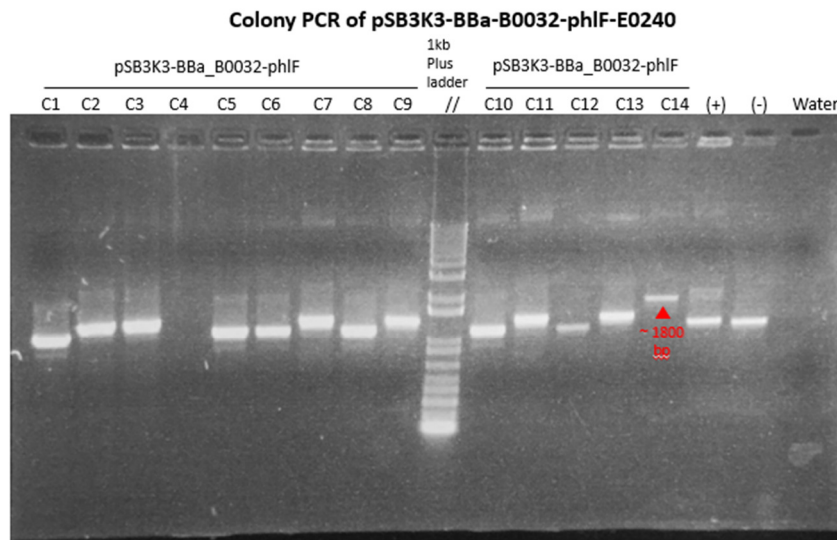
Restriction check of pSB1C3-BBa_B0032-phlF



Restriction check of pSB1C3-BBa_B0032-phlF. Samples were digested with Scal-HF and with XbaI and PstI-HF restriction enzymes. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.41V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 11 (22/08 - 26/08)

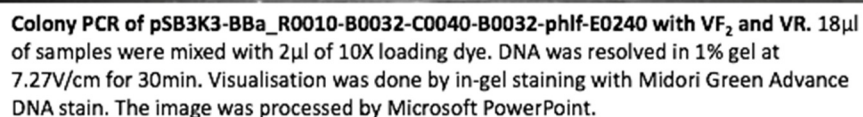
1. Characterization of pSB3K3-BBa_R0010-E0240 (Construct A)
2. Functional assay of Construct A
 - Set 1: Cells are suspended in LB
 - Set 1: Cells are suspended in NaCl
3. Colony PCR of pSB3K3-BBa_B0032-phIF-E0240 (Construct E)
 - Desired band sizes:
 - pSB3K3-BBa_B0032-phIF-E0240: 1822 bp
 - Positive control:
pSB3K3-BBa_J23101-B0032-C0040-B1006-R0040-E0240: 2058
 - Negative control:
pSB3K3-BBa_E0240: 1178 bp



Restriction check of pSB3K3-BBa_B0032-phIF. 25µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the correct band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

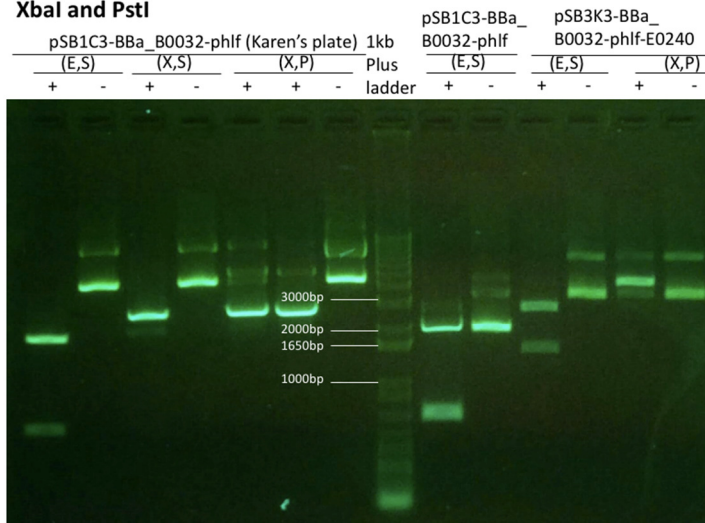
1. Characterization of:

- Colony PCR of pSB3K3-BBa_R0010-B0032-C0040-B0032-phlF-E0240 with VF₂ and VR**



3. Restriction check of pSB1C3-BBa_B0032-PhIF with Enzyme Check
 - Checking the functionality XbaI and PstI

Restriction Check of pSB1C3-BBa_B0032-phIf and Enzyme Check of XbaI and PstI



Restriction Check of pSB1C3-BBa_B0032-phIf and Enzyme Check of XbaI and PstI. 18µl of samples are mixed with 2µl of 5X loading dye. DNA was resolved in 1% gel. Visualisation was done by pre-staining with Midori Green Advance DNA stain. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 13 (05/09 - 09/09)

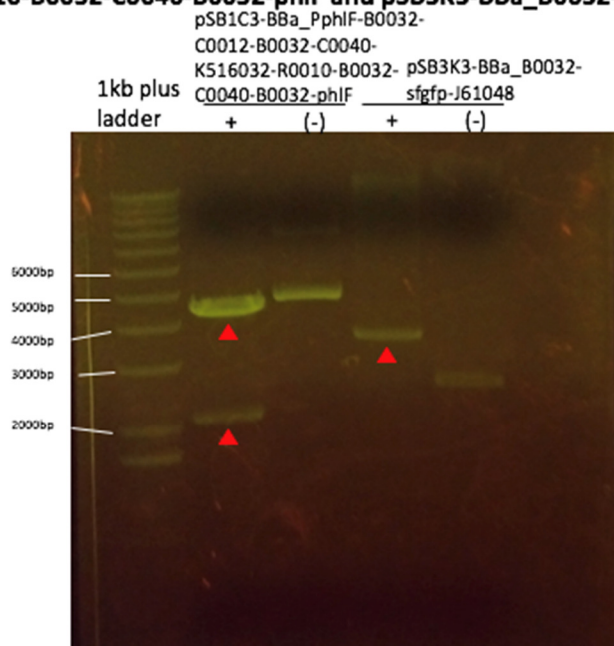
1. Characterization of: pSB3K3-BBa_J23101-B0032-C0040-B1006-R0010-E0240 (Construct C)

Week 14 (12/09 - 16/09)

1. Construction of Plan B Construct

- Digestion of Part 5: PPhIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phlF with EcoRI-HF and SpeI-HF
- Desired band sizes:
Between 4000 bp and 5000 bp
Between 2000 bp and 3000 bp
- Digestion of Part 6: B0032-GFP-J61048 with EcoRI-HF and XbaI
- Desired band sizes:
Between 3000 bp and 4000 bp

Digestion of pSB1C3-BBa_PphIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phlF and pSB3K3-BBa_B0032-sfgfp-J61048



Digestion of pSB1C3-BBa_pPhIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phlF and pSB3K3-BBa_B0032-sfgfp-J61048.

Sample is digested with SpeI-HF, EcoRI-HF and XbaI and EcoRI-HF restriction enzymes. 18µl of samples are mixed with 2µl of 10X loading dye. DNA was resolved in 0.8% gel at 6.41V/cm for 45min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the correct band size. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

2. Colony PCR of Plan B construct, Part 5+6

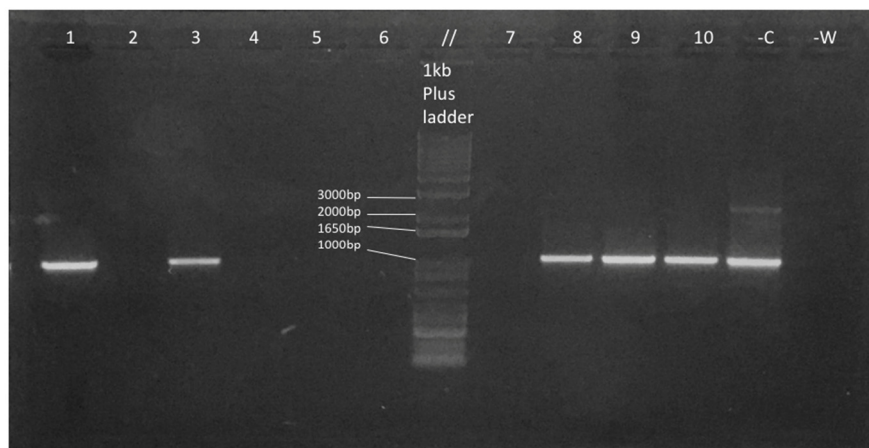
Desired band size: 5556 bp

Negative control:

- pSB3K3-B0032-GFP-J61048: 1107 bp

Colony PCR

pSB3K3-BBa_PPhIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-GFP-J61048



Colony PCR of pSB3K3-BBa_PPhIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-GFP-J61048. 25µl of samples are mixed with 2µl of 5X loading dye. DNA was resolved in 1% gel. Visualisation was done by pre-staining with Midori Green Advance DNA stain. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

4. Characterization of pSB3K3-BBa_R0010-B0032-C0040-B0032-PhIF-E0240 (Construct E)

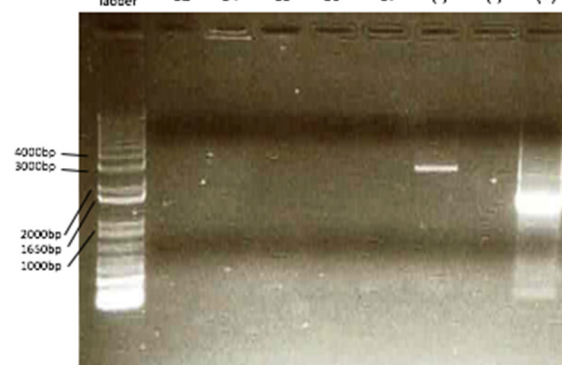
Week 15 (19/09 - 23/09)

1. Colony PCR of Plan B construct, Part 5+6

- Plasmid bandsize: 7992 bp
- Primers use:
- B0032-C0040 FW
- phIF REV
- Desired bandsize: 1348 bp
- pSB1C3-BBa_C0012:
- Plasmid bandsize: 3221 bp
- Amplicon bandsize: 1465 bp

Colony PCR of pSB3K3-BBa_PPhIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048 with B0032-C0040 FW and phIF REV

	pSB3K3-BBa_PPhIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048	pSB1C3-BBa_B0032-K1899003-J61048	No template control	pSB1C3-BBa_C0012				
	C2	C4	C5	C6	C7	(-)	(-)	(+)



Colony PCR of pSB3K3-BBa_PPhIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048 with B0032-C0040 FW and phIF REV. 25µl of samples were mixed with 2.78µl of 10X loading dye. DNA was resolved in 1% gel at 5.78V/cm for 35min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. The image was processed by Microsoft PowerPoint.

2. Colony PCR of Plan B construct, Part 5+6

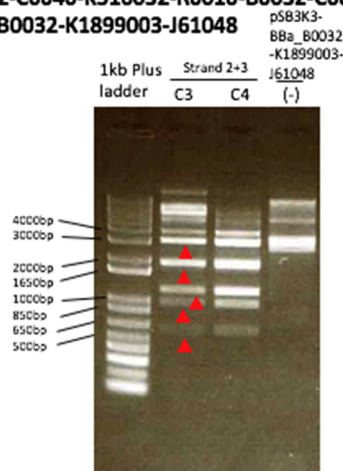
- Plasmid band size: 7992 bp
- Primers use:
- B0032-C0040 FW
- Phlf REV
- Desired band size: 1348 bp

- pSB1C3-BBa_C0012:
- Plasmid band size: 3221 bp
- Amplicon band size: 1465 bp
- Inoculated colony 3 and 4

3. Restriction check of Plan B construct, Part 5+6 with Styl-HF

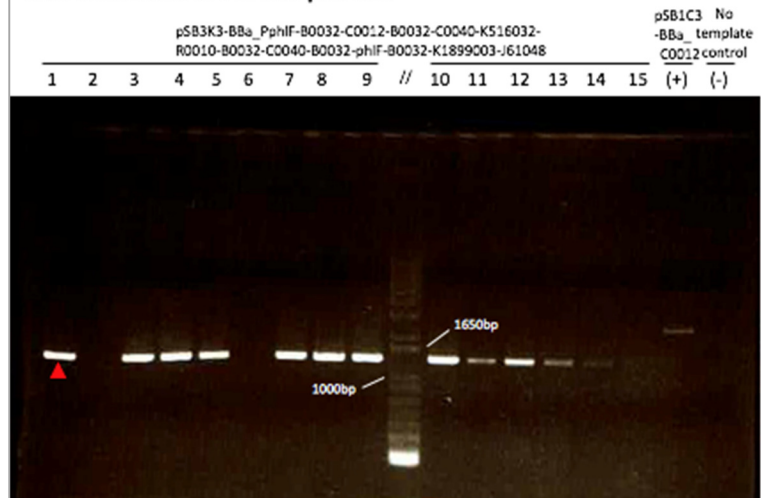
- Plasmid band size: 7992 bp
- Desired band sizes: 3274, 1955, 1196, 973, 594 bp
- Negative backbone control:
Desired band sizes: 3274, 338 bp

Restriction check of pSB3K3-BBa_PphIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048



Restriction check of pSB3K3-BBa_PphIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048. Samples were digested with Styl-HF restriction enzymes. 18µl of samples were mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.67V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the desired band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Colony PCR of pSB3K3-BBa_PphIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048 with B0032-C0040 FW and phlf REV

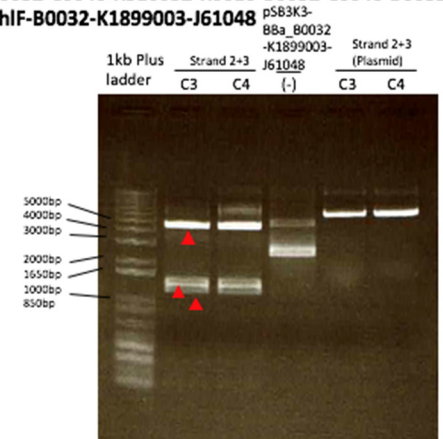


Colony PCR of pSB3K3-BBa_PphIF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048 with B0032-C0040 FW and phlf REV. 25µl of samples were mixed with 2.78µl of 10X loading dye. DNA was resolved in 1% gel at 5.78V/cm for 35min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the desired band size. The image was processed by Microsoft PowerPoint.

4. Restriction check of Plan B construct, Part 5+6 with PvuII

- Plasmid bandsize: 7992 bp
- Desired bandsizes: 4740, 1403, 1243, 336, 153, 24 bp
- Negative backbone control:
- Plasmid bandsize: 3221 bp

Restriction check of pSB3K3-BBa_PhlF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048



Restriction check of pSB3K3-BBa_PhlF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048. Samples were digested with PvuII restriction enzymes. 18µl of samples were mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.67V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the desired band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

Week 16 (26/09 - 30/09)

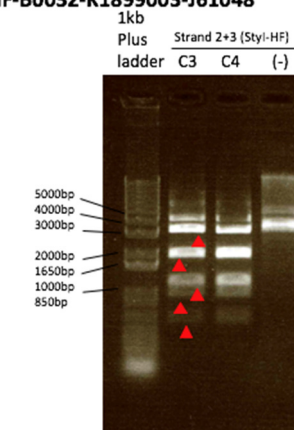
1. Restriction check of Plan B construct, Part 5+6

- Plasmid bandsize: 7992 bp
- Restriction enzyme used: Styl-HF
- Desired bandsizes: 3274, 1955, 1196, 973, 594 bp
- Negative backbone control:
- Desired bandsizes: 3274, 338 bp

2. Colony PCR for Final Construct

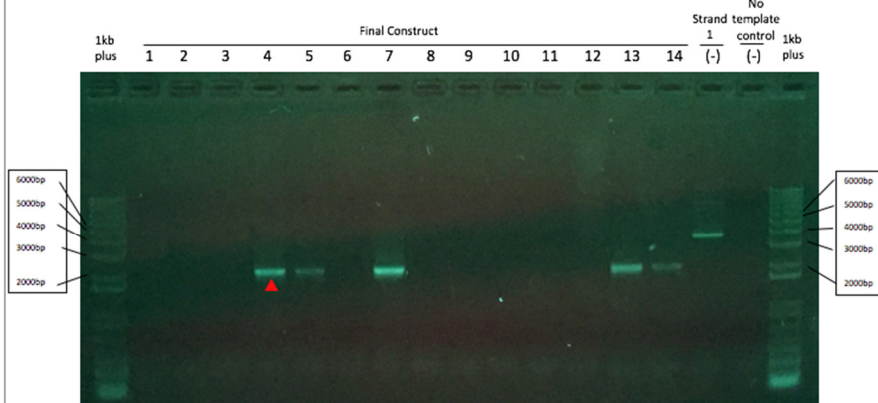
- With Boo32-C0012 FW and oligos-pPhIF REV
- Desired band size: 2043bp

Restriction check of pSB3K3-BBa_PhlF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048



Restriction check of pSB3K3-BBa_PhlF-B0032-C0012-B0032-C0040-K516032-R0010-B0032-C0040-B0032-phIF-B0032-K1899003-J61048. Samples were digested with Styl-HF restriction enzymes. 18µl of samples were mixed with 2µl of 10X loading dye. DNA was resolved in 1% gel at 6.67V/cm for 30min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangles indicate the desired band sizes. The image was processed by Adobe Photoshop and Microsoft PowerPoint.

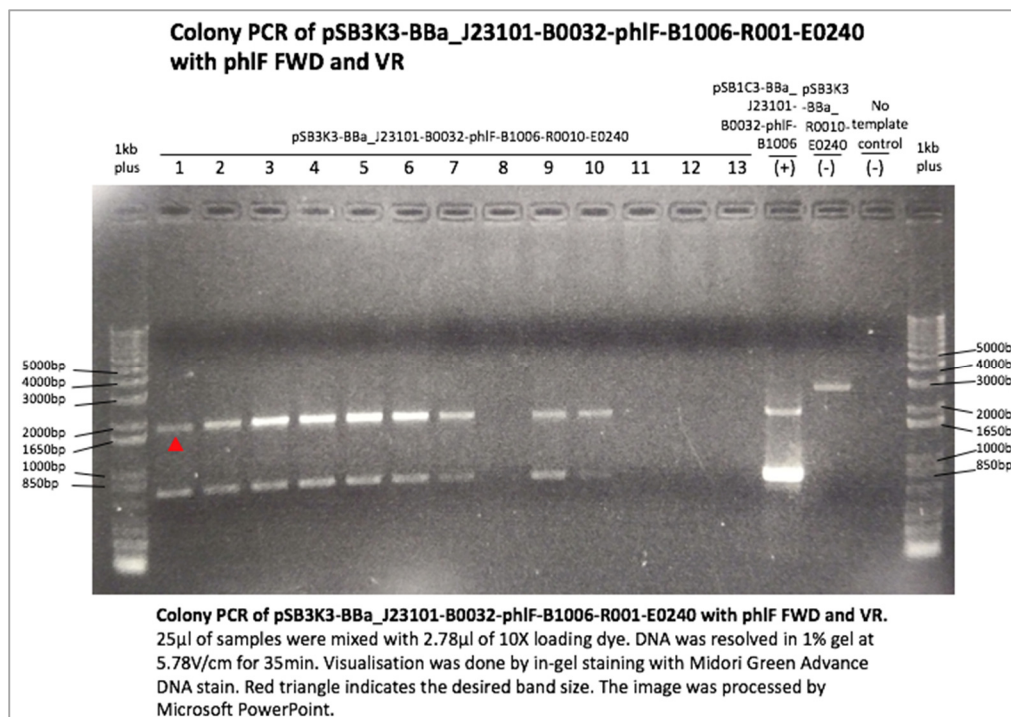
Colony PCR of Final Construct with B0032-C0012 FW and oligos-pPhIF REV



Colony PCR of Final Construct with B0032-C0012 FW and oligos-pPhIF REV. 25µl of samples were mixed with 2.78µl of 10X loading dye. DNA was resolved in 1% gel at 5.78V/cm for 35min. Visualisation was done by in-gel staining with Midori Green Advance DNA stain. Red triangle indicates the desired band size. The image was processed by Microsoft PowerPoint.

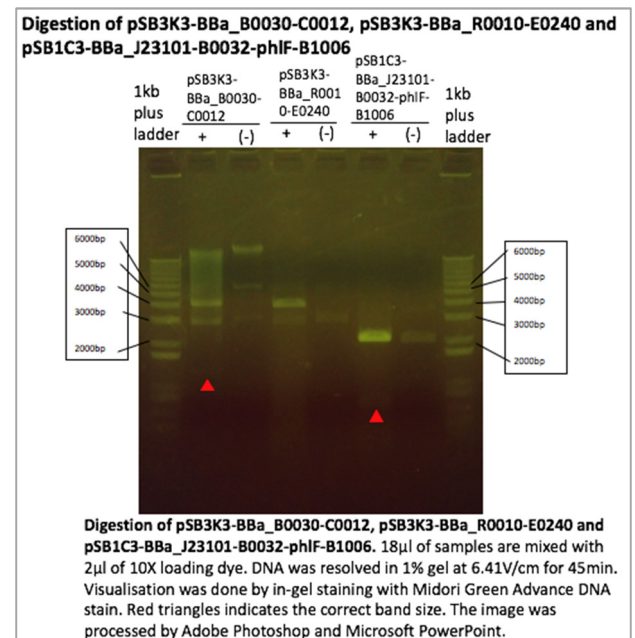
3. Colony PCR for Construct D with PhIF FW and VR

- Expected band size: 1924bp
- Expected band for insert only: 828bp



4. Digestion for Construct D and other parts for submission

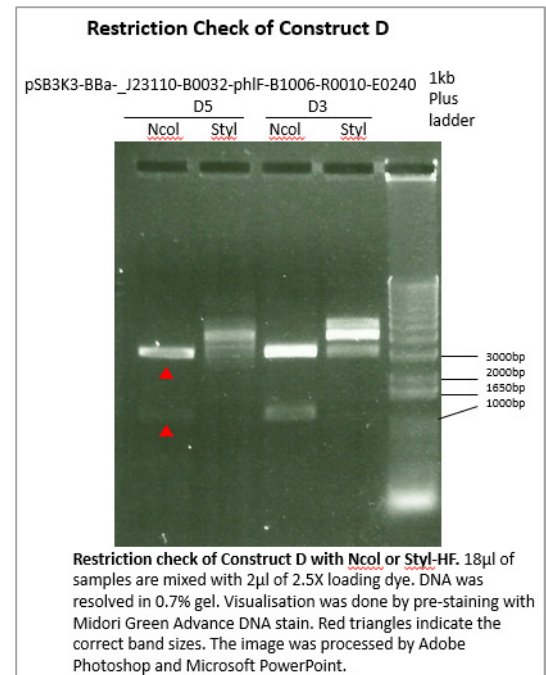
- Expected band size for BBa_B0030-C0012: 1230bp
- Expected band size for pSB3K3-BBa_R0010-E0240: 3819bp
- Expected band size for BBa_J23101-B0032-phIF-B1006: 735bp



Week 17 (3/10 – 7/10)

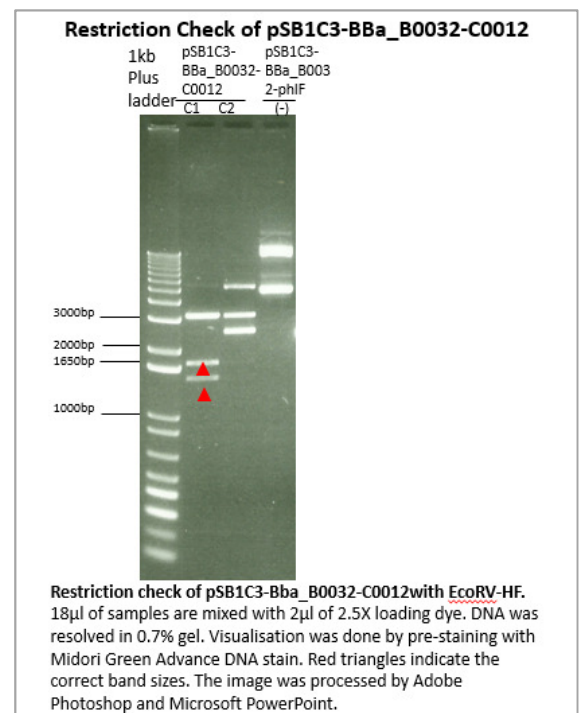
1. Restriction Check for Construct D with NcoI

- Expected band sizes: 3296 bp, 1258 bp



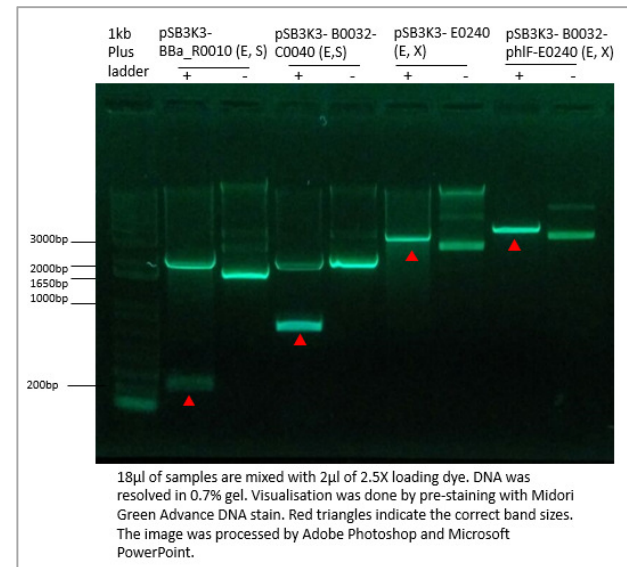
2. For part submission: Restriction Check of pSB1C3-BBa_B0032-C0012 with EcoRV-HF

- Entire plasmid: 3242 bp
- Negative control: pSB1C3-BBa_B0032-phIF
- Expected band sizes: 1498bp, 1746bp
- Negative control: 2692bp



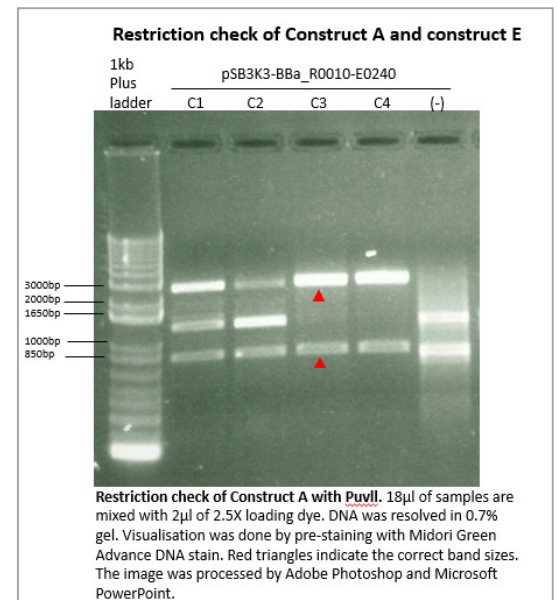
3. Rebuilding Constructs contains *lacp*

- Digestion of:
- pSB3K3-BBa_R0010 (E,S): 223bp
- pSB3K3-BBa_B0032-C0040 (E,S): 727 bp
- pSB3K3-BBa_E0240 (E,X): 3611 bp
- pSB3K3-BBa_B0032-phIF-E0240 (E,X): 4241 bp



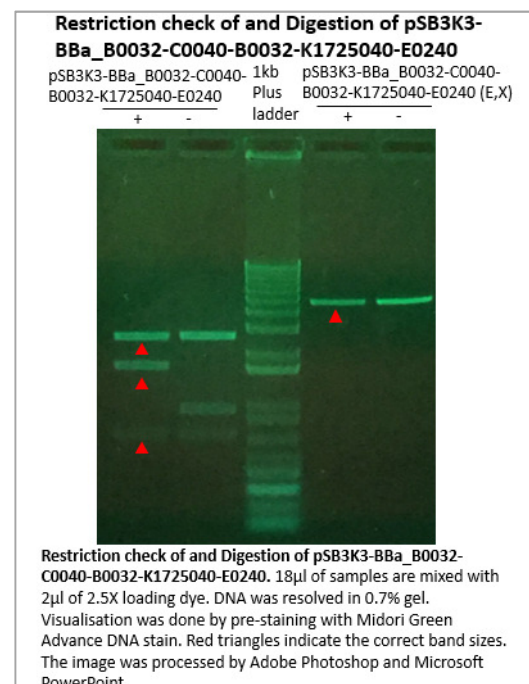
4. Restriction Check of construct A With PuvII

- Negative control: pSB3K3-BBa_E0240
- Expected band sizes: 850 bp, 2980 bp
- Negative control: 3626 bp



5. Restriction Check of pSB3K3-BBa_B0032-C0040-B0032-K1725040-E0240 with NcoI and SpeI-HF:

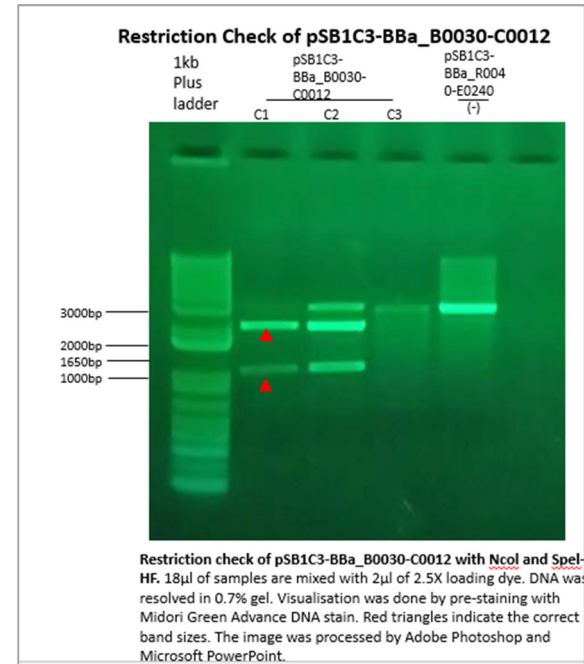
- Negative control: pSB3K3-BBa_B0032-phIF-E0240
- Expected band sizes: 693 bp, 1672 bp, 2603 bp
- Negative control: 693 bp, 960 bp, 2603 bp



Week 18 (10/10 - 17/10)

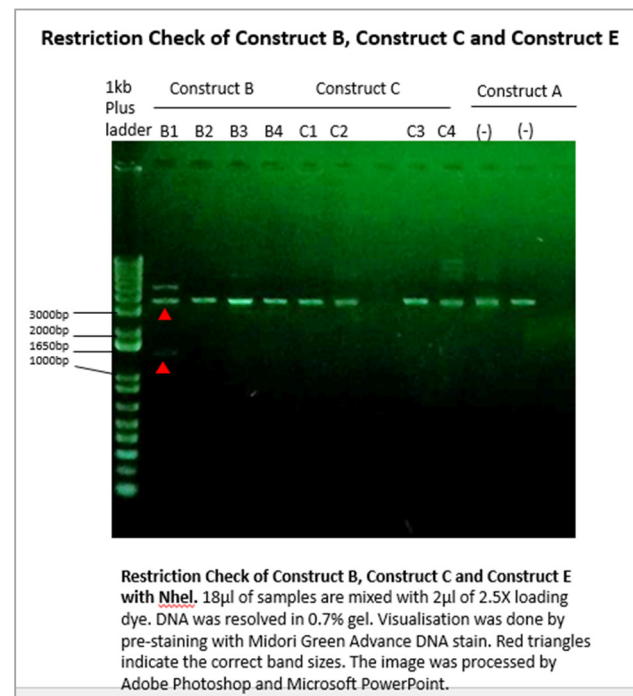
1. For Part Submission: Restriction Check of pSB1C3-BBa_B0030-C0012 with Asel and XbaI

- Negative control: pSB1C3-BBa_R0040-E0240
- Expected band sizes: 2172 bp, 1072 bp
- Negative control: 3008 bp



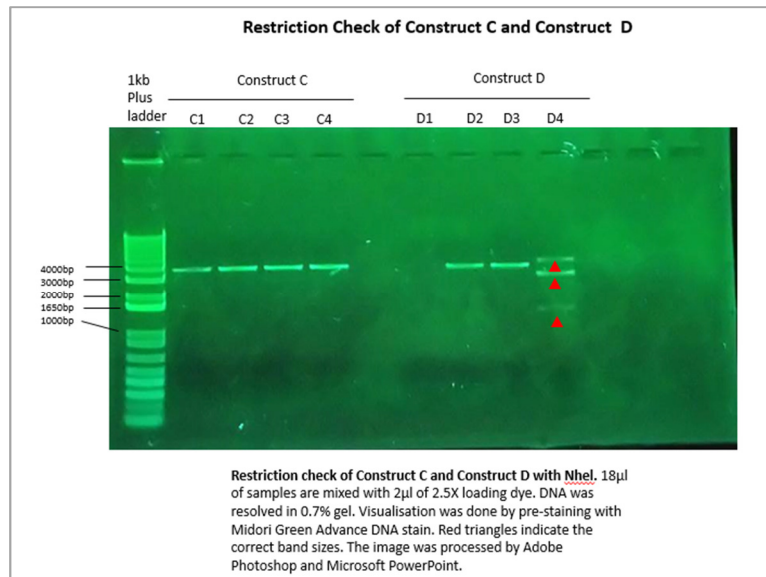
2. Restriction Check of Construct B and construct C with NheI:

- Negative control: pSB3K3-BBa_R0010-E0240
- Desired Band sizes:
- Construct B: 1373 bp, 3721 bp
- Construct C: 1373 bp, 3240 bp
- Negative: 3834 bp



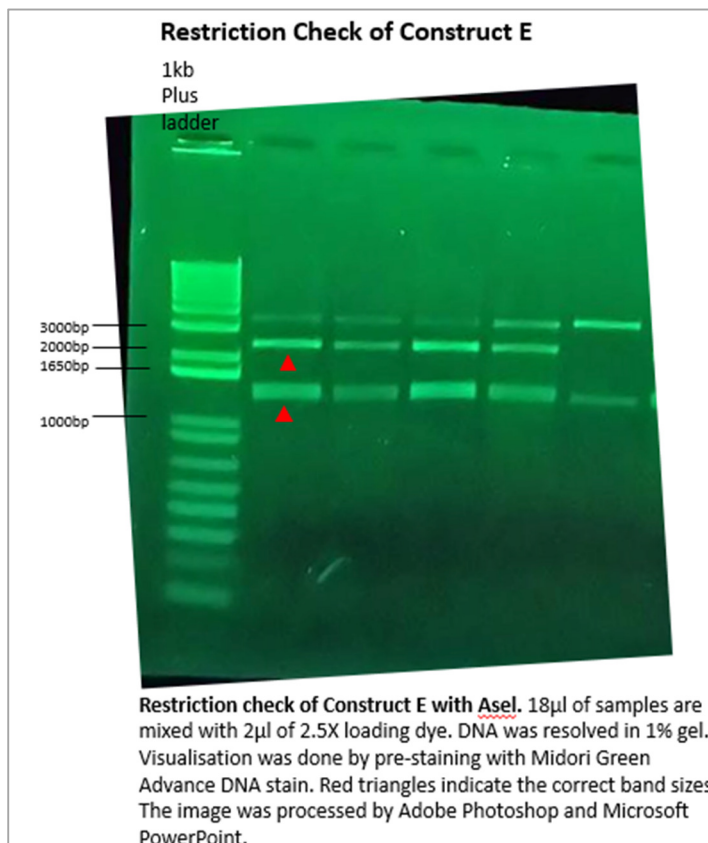
3. Restriction Check of Construct C and D with NheI:

- Desired Band sizes:
- Construct C: 3240 bp, 1373 bp
- Construct D: 3158 bp, 1373 bp



4. Restriction Check of Full construct E with AseI:

- Negative control:
- pSB3K3-BBa_B0032-C0040-B0032-phIF-E0240
- Desired Band Sizes:



- Construct E: 2370 bp, 1336 bp, 1411 bp
- Negative control: 3632 bp, 1336 bp